Data Evaluation Report on the Acute Toxicity of the Formulation GF-2633 (4.38% aminopyralid and 43% 2,4-D dimethylammonium) to Freshwater Invertebrates - *Daphnia magna*

EPA MRID Number 489395-01

Data Requirement:	EPA DP Barcode	D417190
	EPA MRID	489395-01
	EPA Guideline	850.1010

Test material: GF-2633 end-use product
A.I. Aminopyralid **Purity:** 4.38%
A.I. 2,4-D dimethylammonium 43%
Common name: GF-2633
Chemical name: CAS No (aminopyralid). 150114-71-9
CAS No (2,4-D dimethylammonium): 2008-39-1
IUPAC (aminopyralid): 4-amino-3,6-dichloropyridine-2-carboxylic acid
IUPAC (2,4-D dimethylammonium): dimethylammonium (2,4-dichlorophenoxy)acetate
CAS name (aminopyralid): 4-amino-3,6-dichloro-2-pyridinecarboxylic acid
CAS name (2,4-D dimethylammonium): 2-(2,4-dichlorophenoxy)acetic acid compound with *N*-methylmethanamine

Primary Reviewer: Natalie Ross **Signature:** Natalie Ross
Environmental Scientist, CDM Smith **Date:** 06/24/2014

Secondary Reviewer: Teri S. Myers **Signature:** Teri S Myers
Senior Scientist, CDM Smith **Date:** 06/25/2014

Primary Reviewer: Edward Scollon, PhD. **Signature:**
EPA/OPP/EFED/ERB-IV **Date:** 10/02/14 Digitally signed by Scollon, Edward DN: cn=Scollon, Edward, email=Scollon.Edward@epa.gov Date: 2014.10.09 17:36:49 -04'00'

EPA PC Code 005100 (aminopyralid)

Date Evaluation Completed: 10-02-14

CITATION: Rebstock, M. 2011. GF2633: Acute Toxicity to the Water Flea, *Daphnia magna*, Determined Under Static Test Conditions. An unpublished study performed by ABS Laboratories, Inc., Columbia, Missouri. Laboratory report number: 66953. Study sponsored by The Dow Chemical Company. Study initiated on April 13, 2011 and completed on July 25, 2011.

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EXECUTIVE SUMMARY:

The 48-hour acute toxicity of the formulated product GF-2633 (4.38% a.e. aminopyralid in the form of aminopyralid triisopropanolamine, 43% 2,4-D dimethylammonium [35.7% a.e. 2,4-D]) to *Daphnia magna* was studied under static conditions. Daphnids were exposed to GF-2633 at nominal concentrations of 0 (negative control), 6.3, 13, 25, 50 and 100 mg formulation/L (0.28, 0.57, 1.1, 2.2, 4.4 mg aminopyralid/L; 2.7, 5.6, 11, 22, and 43 mg 2,4-D/L) for 48 hr. Mean-measured concentrations were 6.3, 13, 25, 51, 101 mg formulation/L (<LOQ (0.0833), 0.28, 0.58, 1.1, 2.2, 4.4 mg aminopyralid/L; 2.7, 5.6, 11, 22, and 43 mg 2,4-D/L). There was no immobilization in the negative control and no immobilization in any of the treated levels, thus the 48-hr EC_{50} based on immobility is estimated to be >101 mg formulation/L (>4.4 mg aminopyralid/L; >43 mg 2,4-D dimethylammonium/L), the highest nominal concentration tested.

Based on the results of this study, the formulated product GF-2633 would be classified as **practically non-toxic** to *D. magna* up to the highest level tested in this study on an acute toxicity basis, in accordance with the classification system of the U.S. EPA. Relative to the technical grade active ingredient (TGAI) with a 48-hr EC_{50}>98.6 mg a.i./L (MRID 462358-17), the current study of the formulation GF-2633 also resulted in a non-definitive endpoint for aminopyralid (EC_{50}>4,4 mg/L); however, it is not possible to say whether the formulated product is more or less toxic than the TGAI since the formulation was not tested up to the concentrations of aminopyralid evaluated with the TGAI.

This toxicity study is classified as **Acceptable**, scientifically sound and is consistent with guideline recommendations for an acute toxicity study with freshwater invertebrates.

Results Synopsis

Test Organism Age: neonates, <24 hours old
Test Type: Static

48-hr EC_{50}: >101 mg formulation/L (>4.4 mg aminopyralid/L; >43 mg 2,4-D dimethylammonium) 95% C.I.: NA
Probit Slope: N/A
Endpoint(s) affected: None

I. MATERIALS AND METHODS

GUIDELINE FOLLOWED: This study was conducted following procedures outlined in OECD Guideline No. 202 "*Daphnia* Acute Immobilization Test (1984)" and are consistent with OPPTS 850.1010 guidelines.

COMPLIANCE: Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted in compliance with the OECD Principles of GLP (as revised in 1997), ENV/MC/CHEM (98) 17; and the USEPA GLP (1989) Final Rule (40 CFR, Part 160).

A. MATERIALS:

1. Test material Formulation GF-2633
-Aminopyralid triisopropanolamine (TIPA) salt (4.38% a.e.)
-2,4-D dimethylammonium (43% a.i., 35.7% a.e.)

Description: Yellow liquid

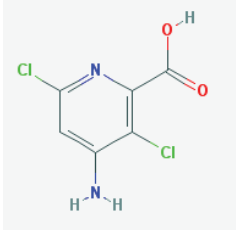
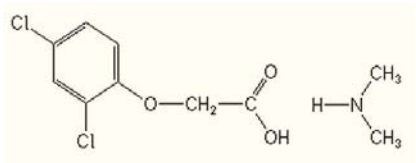
Lot No./Batch No. : F1506-91B

Purity: 4.38% aminopyralid
43% 2,4-D dimethylammonium

Stability of compound Under test conditions: Stable. At termination, concentrations ranged from 99-102% of initial concentrations.

Storage conditions of test chemicals: Ambient temperature in the dark

Physicochemical properties of aminopyralid.

	Aminopyralid		2,4-D	
Parameter	**Values**	**Comments**	**Values**	**Comments**
Water solubility	205 g/L at 20ºC	D415743	5.35 g/L at 25ºC	EPISuite[2]
Vapor pressure at 20°C	7.14×10^{-11} torr at 20°C	D415743	3.98×10^{-8} torr at 25ºC	EPISuite[2]
Structure				
pK_a at 20°C	2.56	D415743	NA	NA
Log K_{ow} at 19ºC; pH 7	-2.87	D415743	0.65	EPISuite[2]

[1] Reregistration Eligibility Decision document http://www.epa.gov/opp00001/chem_search/reg_actions/reregistration/red_G-82_1-Sep-97.pdf

[2] USEPA. 2014. Estimation Program Interface (EPI) Suite. http://www.epa.gov/opptintr/exposure/pubs/episuite.htm

2. Test organism:

Species:	*Daphnia magna*
Age at test initiation:	Neonates, <24 hours old
Source:	Laboratory cultures

B. STUDY DESIGN:

1. Experimental Conditions

a. Range-finding study: A range-finding study was performed using the formulated product GF-1883 at nominal concentrations of 0.010, 0.10, 1.0, 10, and 100 mg formulation/L under static conditions. After 48 hours, no immobilization was observed among daphnids exposed to the treatment levels. Concentrations of 6.3, 13, 25, 50, and 100 mg formulation/L (corresponding to 0.28, 0.57, 1.1, 2.2, and 4.4 mg aminopyralid/L; 2.7, 5.6, 11, 22, and 43 mg 2,4-D dimethylammonium/L) were selected for the definitive exposure.

b. Definitive Study

Table 1: Experimental Parameters

Parameter	Details	Remarks *Criteria*
Parental Acclimation Period: Conditions: (same as test or not) Feeding: Health: (any mortality observed)	None reported Same Fed daily green alga *Pseudokirchneriella subcapitata* (formerly *Selenastrum capricornutum*) None reported	*The recommended acclimation period is a minimum of 7 days. Organisms should not feed during the study. Pretest mortality should be <3% 48 hours prior to testing.*
Duration of the test:	48 hours	*EPA requires 96 hours, except daphnids which are 48 hours.*
Test conditions Static/flow-through: Type of dilution system for flow-through method: Renewal rate for static renewal:	Static N/A N/A	*The recommended flow rates are 5 - 10 volume additions/24 hours; meter systems should be calibrated before and after the study and checked twice daily during the test period.*
Aeration, if any	The dilution water was passed through a sediment filter and aerated prior to use. No aeration was provided to any test chamber during the test.	
Test vessel Material: *(glass/stainless steel)* Size: Fill volume:	 Glass 250 mL 200 mL	*EPA requires: small organisms in 3.9 L (1 gallon) wide mouth jars with 2-3 L of solution or daphnids and midge larvae in 250 ml jars w/ 200 ml fill*
Source of dilution water	Moderately hard freshwater	

Parameter	Details	Remarks *Criteria*
		Recommended source of dilution water is soft, reconstituted water or water from a natural, uncontaminated source. EPA does not recommend the use of dechlorinated tap water; however, its use may be supportable if the biological responses for the organisms and chemical analyses of residual chlorine meet conditions in the Agency's 850.1010 guidelines for dilution water (http://www.epa.gov/opptsfrs/OPPTS_Harmonized/850_Ecological_Effects_Test_Guidelines/Draft/850.1010Opdf). Dilution water should be intensely aerated before the study.

Parameter	Details	Remarks *Criteria*
<u>Water parameters</u> Hardness: pH: Dissolved oxygen: Temperature: Total Organic Carbon: Particulate matter: Metals: Pesticides: Chlorine: Other observations: Intervals of water quality measurement:	 144 mg/L as $CaCO_3$ 8.4 to 8.5 100 to 102% saturation 19.8 to 21°C during study None None None None Not Reported Alkalinity 164 mg/L as $CaCO_3$ Temperature, pH, and dissolved oxygen levels were measured in the control and all test levels at the start and end of the study. Total hardness, alkalinity, and conductivity of the dilution medium were checked prior to use.	 <u>*Hardness*</u>*:* *EPA recommends 40 - 48 mg/L as $CaCO_3$ (OECD recommends 140 - 250 mg/L)* <u>*pH:*</u> *EPA recommends: 7.2 - 7.6 (OECD recommends pH of 6-9); measured at start and end of test in control, high, medium, and low test concentrations* <u>*Temperature*</u>*:* *EPA recommends: 20°C for Daphnia (measured hourly) in at least one test vessel or if water baths are used, every 6 hr, may not vary > 1°C;* OECD *recommends* range of 18-22EC (±1EC) <u>*Dissolved oxygen*</u>*:* *EPA recommends: Measured at start and every 48 hours thereafter in control, high, medium, and low test concentrations.* *Static: 60-100% during 1st 48 hr and 40-100% during 2nd 48 hr* *Flow-through: 60-100% at all times*
<u>Number of replicates</u> Negative control: Solvent control: Treated:	 4 N/A 4 per level	 *EPA requires 2 or more containers for each treatment group; individuals must be randomly assigned to test vessels* *OECD recommends 4 groups of 5 animals for each test concentration and the controls*

Parameter	Details	Remarks *Criteria*
<u>Number of organisms per replicate</u> Negative control: Solvent control: Treatments:	 5 N/A 5 per level	 *EPA/OECD requires 5 treatment levels plus one or more control groups; no more than 10% or 5% of control organisms should die during a static or flow-through study, respectively* *EPA requires a minimum of 20 daphnids in 2 or more containers per treatment; however, if a limit test is conducted, it must be shown that the LC_{50}/EC_{50} is >100 mg/L by exposing ∃ 30 organisms to ≥100 mg/L or greater. Biomass loading rate for static ≤ 0.8 g/L at ≤ 17°C and # 0.5 g/L at > 17°C; flow-through: # 10 g/L at ≤ 17°C and ≤5 g/L at > 17°C.* *OECD recommends a minimum of 20 animals, preferably with 4 groups of 5 animals for each test concentration. There should be at least 2ml of test solution for each animal.*
<u>Treatment concentrations</u> Nominal: Measured:	 0 (negative control), 6.3, 13, 25, 50, and 100 mg formulation/L (equivalent to 0.28, 0.57, 1.1, 2.2, and 4.4 mg aminopyralid/L; 2.7, 5.6, 11, 22, and 43 mg 2,4-D dimethylammonium/L) 0.28, 0.58, 1.1, 2.2, 4.4 mg aminopyralid/L; 2.7, 5.6, 11, 22, and 43 mg 2,4-D dimethylammonium/L	 *Treatment concentrations should include a geometric series of at least five concentrations plus a control with each recommended concentration being at least 60% of the next higher one. The variability of measured concentrations between replicates of the same concentration should not exceed 1.5.* *OECD recommends that the highest test concentration should result in 100% immobilization and not be ≥1 g/L, while the lowest concentration should have no observable effect.*
Solvent (type, percentage, if used)	N/A	

Parameter	Details	Remarks *Criteria*
		Solvents should not exceed 0.5 ml/L for static tests or 0.1 ml/L for flow-though tests. OECD recommends that the solvent not exceed 100 mg/L.
Lighting	16 hrs light/8 hrs dark, with 30 minute transition periods	*EPA-recommended photoperiod is 16 hours of light and 8 hours of dark with a 15-30 minute transition period. OECD: optional light-dark cycle or complete darkness.*
Stability of chemical in the test system	At termination, concentrations ranged from 99-102% of initial.	
Recovery of chemical Level of Quantitation Level of Detection	Mean measured concentrations were 100 to 102% of nominal 0.0833 mg aminopyralid/L Not reported	
Positive control {if used, indicate the chemical and concentrations}	N/A	
Other parameters, if any	None	

2. Observations:

Table 2: Observations

Criteria	Details	Remarks
Parameters measured including the sublethal effects	Mortality (Immobility)	
Observation intervals	24 and 48 hours	

Were raw data included?	Yes	
Other observations, if any	N/A	

II. RESULTS AND DISCUSSION

A. MORTALITY:

According to the study author at 48 hours, mortality was 0% in the control and in the mean-measured 0.28, 0.58, 1.1, 2.4, and 4.4 mg aminopyralid/L (2.7, 5.6, 11, 22, and 43 mg 2,4-D dimethylammonium/L) treatment levels (**Table 3**). Because no treatment level resulted in immobilization, both the NOAEC and EC_{50} values were 4.4 mg aminopyralid/L and >4.4 mg aminopyralid/L. The equates to NOAEC and EC_{50} values for 2,4-D dimethylammonium of 43 and >43 mg a.i./L, respectively.

Table 3: Effect of GF-2633 on Mortality of *Daphnia magna*[a]

Mean measured (and Nominal) Concentrations (µg aminopyralid/L)	No. of organisms	Observation period			
		24 Hours		48 Hours	
		No. Dead	% Mortality	No. Dead	% Mortality
Control	20	0	0	0	0
0.28 (0.28)	20	0	0	0	0
0.58 (0.57)	20	0	0	0	0
1.1 (1.1)	20	0	0	0	0
2.2 (2.2)	20	0	0	0	0
4.4 (4.4)	20	0	0	0	0
NOAEC	4.4 mg aminopyralid/L 43 mg 2,4-D dimethylammonium/L				
LC_{50}	>4.4 mg aminopyralid/L; >43 mg 2,4-D dimethylammonium/L				

B. SUB-LETHAL TOXICITY ENDPOINTS:

Daphnids were only examined for immobility.

C. REPORTED STATISTICS:

No concentration tested resulted in immobilization; therefore, statistical analysis was not performed.

D. VERIFICATION OF STATISTICAL RESULTS:

Statistical Method: Statistical analyses were not conducted, as immobility was not observed in this study; the EC_{50} was empirically estimated to be higher than the highest test level.

48-hr LC_{50}: >101 mg formulation/L (>4.4 mg aminopyralid/L; >43 mg 2,4-D dimethylammonium/L) 95% C.I.: NA
Probit Slope: N/A
Endpoint(s) affected: None

E. STUDY DEFICIENCIES:

No major deficiencies from the OPPTS 850.1010 guidelines were observed.

F. REVIEWER'S COMMENTS:

The reviewer agreed with the study author; no toxicity was observed in this study.

G. CONCLUSIONS:

This toxicity study is classified as **Acceptable**, scientifically sound and is consistent with guideline recommendations for an acute toxicity study with freshwater invertebrates. At 48 hours, mortality was 0% in the control and in the mean-measured 0.28, 0.58, 1.1, 2.4, and 4.4 mg aminopyralid/L treatment levels. Because no treatment level resulted in immobilization, the LC_{50} value was >4.4 mg aminopyralid/L.

48-hr LC_{50}: >101 mg formulation/L (>4.4 mg aminopyralid/L; >43 mg 2,4-D dimethylammonium/L)
95% C.I.: NA

Probit Slope: N/A

III. REFERENCES:

None